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RESEARCH ARTICLE

Growth, lipid, and pigment properties of locally isolated (Kastamonu, Türkiye) *Chlorella* sp.

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ABSTRACT

Chlorella has become one of the most studied and produced microalgae, with *Spirulina* among the hundreds of species since the beginning of microalgal biotechnology. The growth performance of microalgae and the biochemical composition of the biomass may also vary significantly by strain. Therefore, it is thought that searching for new strains from aquatic environments is important in providing the most suitable microalgae for production. An isolated strain from Daday Stream was cultured in the laboratory at Kastamonu University. BG-11 was used as a medium, and CO_2 from the air was used as a carbon source in the experiments. The initial cell number was arranged to 1.0×10^6 cells $mL^{-1,}$ and the highest cell number was found on the 17th day as 40.52×10^6 cells mL^{-1}. Chlorophyll *a* and carotenoids were determined at the end of the experiment and were found as 3.48±0.08 µg mL^{-1} and 1.16±0.02 µg mL^{-1}, respectively. Total lipid amount and fatty acid composition analysis were also conducted at the end of the study. According to the analyses, the lipid content of *Chlorella* sp. was found to be 15.37±0.00% (w/w). ΣSFA (saturated fatty acid), ΣMUFA (monounsaturated fatty acid), and ΣPUFA (polyunsaturated fatty acid) ratios were calculated to be 31.30±1.21%, 4.99±0.34% and 63.71±2.65%, respectively.

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Introduction

Natural sources have decreased considerably since the increase in human population and industrial production. Water resources provide an excellent habitat for microalgae growth and proliferation. However, studies on the taxonomy and diversity of these group of microorganisms are still limited. Microalgae stand out with their large-scale producibility and usability in different industrial fields such as aquaculture, nutrition, pharmaceuticals, and even energy (Shah et al., 2018; Durmaz et al., 2020; Mehariya et al., 2021).

Chlorella has become one of the most studied and produced microalgae, with *Spirulina* among the hundreds of species since the beginning of microalgal biotechnology (Sugiharto, 2020). Lutein production, used as a food additive, biodiesel-oriented studies, and environmental applications constitute the main topics of this species (Farooq et al., 2015; McClure et al., 2019; Asadi et al., 2019; Konar et al., 2022).

Alteration of the biochemical composition of microalgae by culture conditions is already known for decades (Renaud et al., 1991; Ram et al., 2019). New studies are carried out every day for this purpose. Searching for more efficient production of microalgae to obtain target metabolites at higher concentrations causes these efforts. However, this is not the only way to achieve the desired production biochemical composition. The growth performance of microalgae and the biochemical composition of the biomass may also vary significantly by strain. Therefore, it is thought that searching for new strains from aquatic environments is crucial in providing the most suitable microalgae for production. This study was aimed to examine isolate and biochemical composition of new strain from the Daday Stream.

Material and Methods

The water sample was collected from the Daday Stream (41°27'18.4" N 33°42'15.2" E) in Kastamonu, Türkiye. The Autoclaved (121°C, 15 mins) falcon tubes were used for that purpose. First, the samples were inoculated into petri dishes on the same day. After several repeats of the inoculation process, an isolated microalgae strain was obtained. The strain was inoculated to the tubes and flasks to secure appropriate stock.

The culture growth performance and lipid content of the isolated strain were investigated and, for this purpose BG-11 was used as a medium, and cultures were mixed with air. 0.2 µm syringe filters were used in aeration to avoid contamination. The temperature was 21°C during the experiment period. Cultures were illuminated with 40 µmol.m^{-2}.s^{-1} fluorescent lamps (Apogee MQ-620). Taxonomic identification was made according to Vuuren et al. (2006) and Bellinger & Sigee (2010).

Growth

The experiment and the analysis were completed in triplicate. Cell numbers were counted under the light microscope daily. The specific growth rate (µ) was calculated according to the formula given in the Eq. (1): (*X*: cell number; *t*: time).

$$\mu = \frac{\ln X_2 - \ln X_1}{t_2 - t_1} \quad (1)$$

Cell size was also measured. Cell size measurement was conducted with ImageJ (National Institutes of Health, USA) software by using digital images of microalgae samples on a Neubauer hemocytometer (Figure 1).

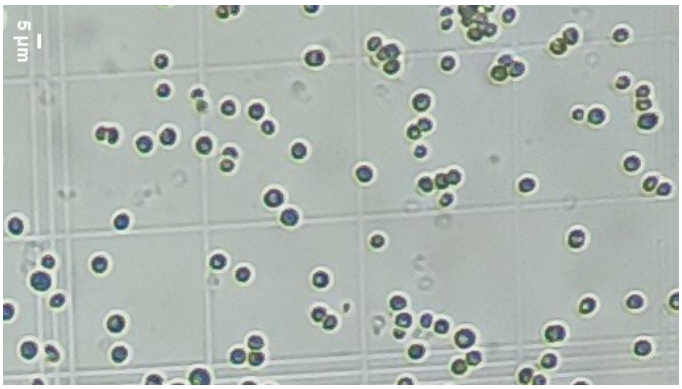

Figure 1. *Chlorella* sp. (Daday Stream) cell size on Neubauer hemocytometer

Dry weights were determined at the end of the experiment. 0.45 µ Whatman filters were weighed after drying at 105°C for 2 h. Then, 5 mL samples from each culture were filtered and dried at the same temperature until the weights became constant and were weighted.

Pigments

Chlorophyll *a* and carotenoids were determined spectrophotometrically. 5 mL samples were centrifuged, and supernatants were discarded. After adding 5 mL methanol, samples were mixed by vortex and placed in ultrasound for 15 mins. Lastly, samples were centrifuged again, and supernatants were used to determine absorbance values. Pigment amounts were calculated using the formulas below (1-2).

(i) Chlorophyll a (µg mL^{-1}) = 13.9 A_{665} (Macías-Sánchez et al., 2005)

(ii) Carotenoids (µg mL^{-1}) = 4.5 A_{470} (Zou & Richmond, 2000)

Lipids

SPV (sulfo-phospho-vanillin) method (Mishra et al., 2014) was used to determine the lipid content in addition that, fatty acids analysis was conducted according to the direct transesterification method (Chu et al., 2015).

Results

After the isolation process, the obtained strain was examined under the microscope. The size of the cells varied between 4.42-8.32 µm, and the average cell size was calculated as 5.90±1.00 µm. Cells were single and non-motile. Also, single-cell formation was observed without flagella. According to the visual identification, the strain was determined to be *Chlorella* sp. (Vuuren et al., 2006; Bellinger & Sigee, 2010).

Growth

Cell numbers were counted daily for 18 days of the experiment period. The initial cell number was arranged to 1.0×10^6 cells mL^{-1}; the highest cell number was found on the 17th day as 40.52×10^6 cells mL^{-1} (Figure 2). The specific growth rate reached the highest point at the beginning of the experiment (4^{th} day) and was calculated as 1.08±0.06. When specific growth was turned negative at the 18^{th} day, the experiment was terminated. Dry weight analysis was conducted on the 18^{th} day of the experiment and found 2.47±0.15 g L^{-1}. According to the cell number of the day, cellular weight was calculated as 60.15±3.83 pg $cell^{-1}$.

Pigments & Lipid

Chlorophyll *a* and carotenoids were determined at the end of the experiment and were found as 3.48±0.08 µg mL^{-1} and 1.16±0.02 µg mL^{-1}, respectively. Total lipid amount and fatty acid composition analysis were also made at the end of the study. According to the results, the lipid content of *Chlorella* sp. (Daday Stream) was found to be 15.37±0.00% (w/w).

The direct transesterification method was chosen to determine the local strain's fatty acid composition. ΣSFA (saturated fatty acid), ΣMUFA (monounsaturated fatty acid), and ΣPUFA (polyunsaturated fatty acid) ratios were found to be 31.30±1.21%, 4.99±0.34% and 63.71±2.65%, respectively (Table 1).

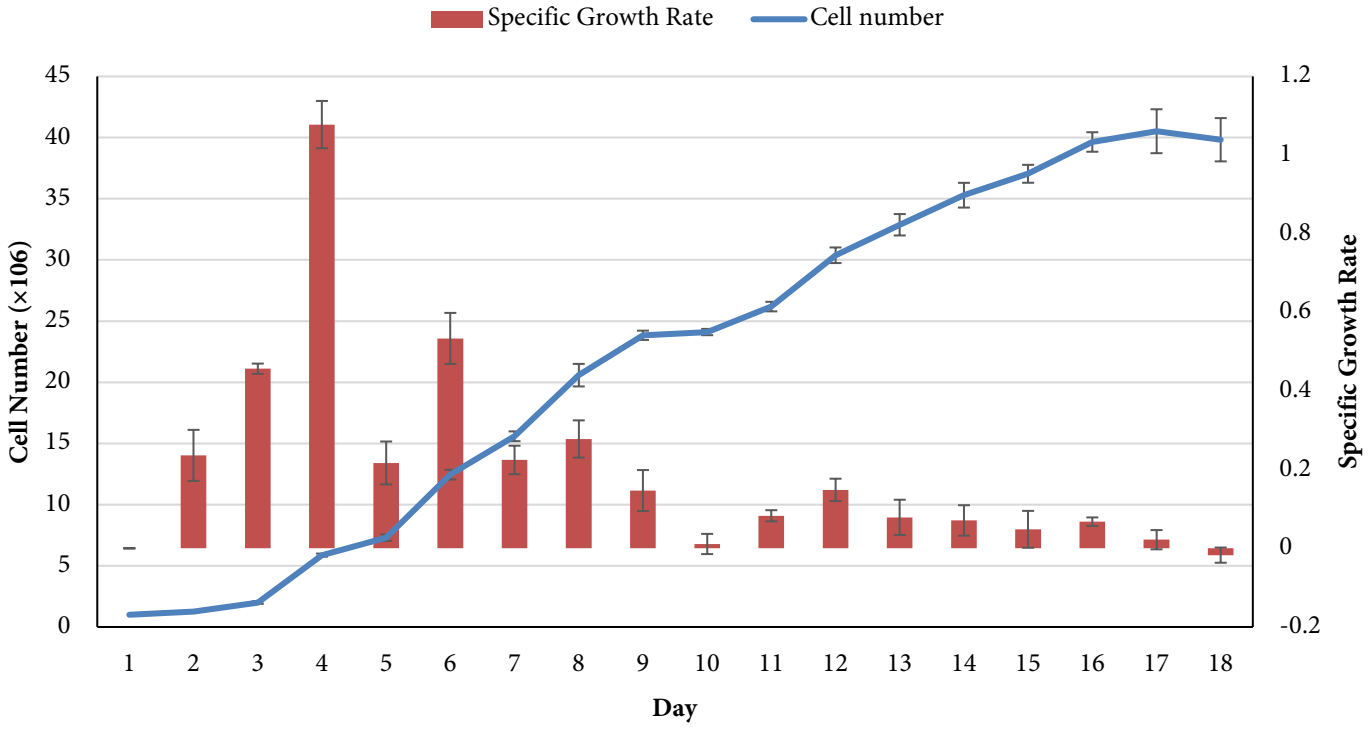

Figure 2. Growth parameters of *Chlorella* sp. (Daday)

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Table 1. Fatty acid composition of *Chlorella* sp. (Daday Stream)

Fatty Acids	Composition (%)
Octanoic acid (C8:0)	0.00±0.00
Decanoic acid (C10:0)	0.00±0.00
Dodecanoic acid (C12:0)	0.12±0.04
Tridecanoic acid (C13:0)	0.00±0.00
Tetradecanoic acid (C14:0)	0.74±0.02
Palmitic acid (C16:0)	29.03±0.96
Palmitoleic acid (C16:1)	0.12±0.03
Stearic acid (C18:0)	0.69±0.02
Oleic acid (C18:1)	4.87±0.31
Linoleic acid (C18:2)	52.89±1.78
Eicosanoic acid (C20:0)	0.17±0.05
α-linolenic acid (C18:3)	10.71±0.84
cis-11-Eicosenoic acid (C20:1)	0.00±0.00
Heneicosanoic acid (C21:0)	0.14±0.04
Docosanoic acid (C22:0)	0.16±0.04
11,14,17-Eicosatrienoic acid (C20:3)	0.12±0.03
Arachidonic acid (C20:4)	0.00±0.00
Tricosanoic acid (C23:0)	0.25±0.04
ΣSFA	**31.30±1.21**
ΣMUFA	**4.99±0.34**
ΣPUFA	**63.71±2.65**

Note: SFA; saturated fatty acids, MUFA; monounsaturated fatty acids, PUFA; polyunsaturated fatty acids.

Discussion

Chlorella is one of the most common species found in freshwater. As stated above, the current strain isolated from the Daday Stream was cultured under laboratory conditions. 2.47±0.15 g L^{-1} dry weight was obtained in this study with 60.15±3.83 pg $cell^{-1}$ cellular weight. The cell weight of *Chlorella vulgaris* is given as 6-18 pg $cell^{-1}$ in the study on the effect of different culture media on growth (Chia et al., 2013). Even if it is stated that cellular weight is alterable by culture nutrient composition, *Chlorella* sp. (Daday Stream) cell weight is higher than that. In another study, *Chlorella vulgaris* culture with modified BG-11 provided 0.9±0.001 g L^{-1} biomass after 12 days (Wong et al., 2017). Results of the study on 5 different *Chlorella* strains and their biomass production with 4 different culture media show that both the strain and the culture medium are effective in growth performance (Sharma et al., 2016). Since the dry weights varied between 0.9-1.7 g L^{-1} in the mentioned studies, it can be stated that the *Chlorella* sp. (Daday Stream) strain exhibited higher growth performance.

Chlorella is known as a source of chlorophylls, lutein, astaxanthin, and a few other carotenoids with a 1-2% (w/w) pigment ratio of total (Safi et al., 2014). In this study, chlorophyll *a* and carotenoids of *Chlorella* sp. were found as 3.48±0.08 μg mL^{-1} and 1.16 μg mL^{-1}, respectively. It was reported that *Chlorella* strains isolated from Nigeria contain similar amounts of chlorophyll *a* and carotenoids varied 1-3.5 μg mL^{-1} and 0.4-1.2 μg mL^{-1}, respectively (Idenyi et al., 2021). However, much higher pigment contents are also reported in several studies. For instance, 2.8% total pigments for *Chlorella* sp. (Erbil et al., 2021), 6.04% of pigment concentration for *Chlorella vulgaris* (Mastropetros et al., 2022), and 9.33% pigment concentration for another *Chlorella vulgaris* strain (Soto-Ramirez et al., 2021) are indicated.

The lipid ratio and fatty acid composition of microalgae are also alterable by culture medium and strain. For instance, it is indicated that *Chlorella* sp. 1 strain's lipid accumulation varied between 14-16% depending on the medium. Also, lipid accumulation of another strain (*Chlorella* sp. 2) was given between 8-10% in the same study (Sharma et al., 2016). *Chlorella* sp. T4 can accumulate 25.87% lipid, another strain from South Africa. This result is higher than the lipid ratio of *Chlorella* sp. (Daday sample). However, the T4 strain with 0.77 g L^{-1} biomass production is three times lower than *Chlorella* sp. (Daday sample), which leads to lower lipid production (Gumbi et al., 2022).

In the study, dominant fatty acids of *Chlorella* sp. were palmitic acid (29.03%), linoleic acid (52.89%), and α-linolenic acid (10.71%). Palmitic acid, around 30%, is a widespread result for *Chlorella* species (Sharma et al., 2016; Dahiya et al., 2021; Gumbi et al., 2022). Previous studies conducted with *Chlorella* show that linoleic acid is highly variable and can be found between 13.6 and 42.54% (Tang et al., 2011; Ördög et al., 2016; Gumbi et al., 2022). *Chlorella* sp. (Daday sample) was determined as one of the richest linoleic acid sources among the *Chlorella* strains. α-linolenic acid is another fatty acid found in *Chlorella* with different ratios. For instance, studies on *Chlorella vulgaris* differ with the α-linolenic acid ratio, which varies between 8.3-27.5% (Yusof et al., 2011; Park et al., 2014). We found that *Chlorella* sp. contains 10.71% α-linolenic acid. This ratio can be accepted as an average value considering various strains from previous studies (Zhu et al., 2015; Krzeminska et al., 2015; Ördög et al., 2016; Anto et al., 2019).

Conclusion

According to the result of this study, the locally isolated *Chlorella* sp. strain showed promising growth performance. Lipid and pigment accumulation ratios were also acceptable if it was considered that any optimization or stress conditions were not applied. Further investigations may help to improve

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the growth and desired biochemical composition of the *Chlorella* sp. strain.

Compliance With Ethical Standards

Authors' Contributions

ME: Project administration, Methodology, Funding acquisition, Writing – review & editing

YD: Investigation, Methodology, Formal analysis, Writing – original draft, Writing – review & editing

GÇE: Investigation, Methodology, Formal analysis, Data curation

All authors read and approved the final manuscript.

Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical Approval

For this type of study, formal consent is not required.

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Data Availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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